L15: Entry 7 of 10 File: USPT Aug 4, 1987

DOCUMENT-IDENTIFIER: US 4684615 A
TITLE: Stabilized isoenzyme control products

BSPR:
Isoenzymes, or isozymes, as they are alternatively referred to, are enzymes in multiple forms which are capable of performing the same general function but at different rates. They are sufficiently different in chemical composition so that they are generally separable electrophoretically. One such isoenzyme, lactate dehydrogenase (LDH) is found in five electrophoretically distinct fractions. Each of these electrophoretic species of LDH is a tetramer consisting of two polypeptide chain units, H and M, present in different proportion: H.sub.4, MH.sub.3, M.sub.2 H.sub.2, HM.sub.3, and M.sub.4. These five isoenzymes differ in catalytic activity (affinity for the substrate, pyruvate as measured by the Michaelis constant), amino acid composition, heat lability, and immunological responses. The two peptides H and M are coded by different genes. Thus the type of enzyme present is under genetic control and regulated by the conditions of the environment imposed upon the cell.

BSPR:
In accordance with the objects and principles of the present invention, isoenzyme control reagents are provided for creatinine kinase, lactate dehydrogenase, alanine aminotransferase and aspartase aminotransferase which are substantially stabilized by the addition of plexiform stabilizing means. The preferred plexiform stabilizing means is selected from the group consisting of maltose, mannitol, cellobiose and lactose with the latter most being the most preferred. The plexiform stabilizing means is advantageously provided in a final concentration range of about 2%-8% with the ideal concentration occurring at about 6%. The ideal isoenzyme control reagent will have substantially all water removed, such as by lyophilization, to assist in long term storage and stability.

DEPR:
Enzymes which can be stabilized as described here include oxidoreductases, transferases, dehydrogenases, transaminases, peptidases, hydrolaser, isomerases, mutases and the like. In addition to enzymes already described, other enzymes which could be stabilized herein include but are not limited to malate dehydrogenase; creatine kinase (CK, CPK); alkaline phosphatase (AP, ALP, ALK-phos); aspartate aminotransferase (AST, OT, GOT); alanine aminotransferase (ALT, PT, SGPT); gamma glutamyl transpeptidase (gammaGT, gammaGTP); glutamic oxalacetic transaminase (SGOT); glutamic pyruvic transaminase (GTP); alpha amylase; beta amylase; lactate dehydrogenase (LD, LDH, lactic dehydrogenase); glucose-6-phosphate dehydrogenase (G6PDH); hexokinase (HK); glucose dehydrogenase; glucose oxidase; peroxidase (HRP, HPO, HRPO, PO); glycerol dehydrogenase; glutamate dehydrogenase, cholesterol oxidase; cholesterol esterase; lipase; uricase; urease; glycerol kinase; aldolases; synthetases; nucleases; polymerases; and the like.

DEPR:
The polymer herein can also be used to synthesize peptide bonds. Typically, equimolar solutions containing modified amino acid(s) are dissolved in a solvent system containing an organic solvent such as dioxane or THF (tetrahydrofuran), or a tertiary amine such as (iPr).sub.2 NEt, to form an amino acid mixture. More preferably, the organic solvent is acetonitrile. The solutions dissolved in the solvent system are defined herein as amino acid acceptor and amino acid donor solutions. Examples of such solutions can be found in Table 2 of Example 10.

DEPR:
A conjugated enzyme such as protease (the enzyme incorporated into one of the compounds of the invention) may be added to the amino acid mixture in an amount of about 10.sup.-6 to about 10.sup.-1 mole fraction relative to the amount of amino acid. The conjugated enzyme can be prepared as in Example 8. The reaction is performed at from about 0.degree. to 70.degree. C., at about 1 to about 24 hours. The enzyme polymer conjugate is removed from the formed peptide by filtering and the solvent is evaporated to obtain the resulting peptide.

DETL:
TABLE 2

Enzyme (Solvent, Time) Acceptor Donor Isolated (HPLC) at 23.degree. C. Amino Acid Amino Acid Product Yield (%)

CPC-CT AcPhe-OEt Ala-NH.sub.2 AcPheAla-NH.sub.2 97(100) (Dioxane, 12 h) CPC-CT * AcPhe-OEt Ala-NH.sub.2 AcPheAla-NH2 94(98) (THF, 12 h) CPC-BPN' CbzLeuLeu-OMe Leu-O.sup.t Bu CbzLeuLeuLeu-O.sup.t Bu 68(95) (Acetonitrile, 22 h) CPC-BPN' CbzLeuLeu-OMe PheLeu-O.sup.t Bu CbzLeuLeuPheLeu-O.sup.t Bu 92(98) (Acetronitrile, 24 h) CPC-BPN' CbzValLeu-OMe PheLeu-O.sup.t Bu CbzValLeuPheLeu-O.sup.t Bu 95(98) (Acetronitrile, 24 h) CPC-BPN' CbzValLeu-OMe Ala-NH.sub.2 CbzValLeuAla-NH.sub.2 65(95) (Acetronitrile, 24 h) CPC-BPN' CbzLeuLeu-OMe Ala-NH.sub.2 CbzLeuLeuAla-NH.sub.2 55(90) (Acetronitrile, 24 h) CPC-T CbzPhe-OH Leu-OMe CbzPheLeu-OMe 65(95) (Acetronitrile, 24 h)

* at 50.degree. C.

L8: Entry 67 of 71 File: USPT May 22, 1984

DOCUMENT-IDENTIFIER: US 4450232 A
TITLE: Incorporation of pyridoxal phosphate in dry analytical elements for the determination of enzymes

Brief Summary Text (2):
The present invention relates to dry analytical elements which are useful in determining the amount of pyridoxal phosphate activated enzyme in a liquid sample. The elements of the present invention are particularly useful in quantitating aspartate aminotransferase and alanine aminotransferase enzymes.

Brief Summary Text (4):
Transaminase enzymes are enzymes which catalyze the transfer of an .alpha.-amino group from an .alpha.-amino acid to an .alpha.-keto acid. These enzymes are also sometimes referred to as aminotransferases. Two of the most clinically significant aminotransferase enzymes are L-alanine:.alpha.-ketoglutarate aminotransferase, EC 2.6.1.2 (commonly referred to as alanine aminotransferase or ALT) and L-aspartate:.alpha.-ketoglutarate aminotransferase, EC 2.6.1.1 (commonly referred to as aspartate aminotransferase or AST) (including mitochondrial and cytoplasmic isoenzymes).

Brief Summary Text (11):
Because of the importance of pyridoxal phosphate on the activation of the apo-enzyme form of AST and ALT, this activation has been widely studied. For example, it is known that in dilute solution, activation of the apo-enzyme occurs at low concentrations of pyridoxal phosphate. Thus, the IFCC recommends that the incubation be carried out in a 0.1 mmole per liter pyridoxal phosphate solution. Other studies have indicated that somewhat higher levels of pyridoxal phosphate might be desirable, for example, up to 0.3 mmole per liter. However, in solution, additional pyridoxal phosphate gave little, if any, further activation. In addition, because of the spectral absorption of pyridoxal phosphate and the possible inhibition of the coupling enzyme used in the indicator reaction of the assay, higher concentrations of pyridoxal phosphate are not used in solution assays (see Hafkenscheid et al, "Influence of Pyridoxal-5'-phosphate on the Determination of the Alanine Aminotransferase and Aspartate Aminotransferase of Commercial Test Sera", J. Clin. Chem. Clin. Biochem., Vol. 17, pp. 219-223, (1979); Soo-se Chen et al, "Modification of Pig M.sub.4 Lactate Dehydrogenase by Pyridoxal 5'-Phosphate", Biochem J., Vol. 149, pp. 107-113, (1975) and "Reversable Modification of Pig Heart Mitochondrial Malate Dehydrogenase by Pyridoxal 5'-Phosphate", Biochem. J., Vol. 151, pp. 297-303 (1975)).

Detailed Description Text (2):
The present invention is described in relation to dry analytical elements for the determination of AST and ALT. It will be understood, however, that other enzymes which are activated by pyridoxal phosphate are quantitated by elements containing a high level of this activator. By choice of suitable detecting reagents, enzymes such as other transaminases, for example glutamate-cysteine transaminase, and other transaminases described in table 16-4 on page 684 of Mahler et al, Biological Chemistry, Harper and Row, 1966; isomerases, for example isomers for analine, glutamate, proline, lysine and serine and dicarboxylases, for example those described in Mahler et al, cited above, at page 685, are determined.

Other Reference Publication (4):

Hafkenscheid et al. "Influence of Pyridoxal-5'-phosphate on the Determination of the Alanine Aminotransferase and Aspartate Aminotransferase of Commercial Test Sera", J. Clin. Chem. Clin. Biochem., vol. 17, pp. 219-223 (1979).

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L15: Entry 5 of 10 File: USPT Feb 20, 1996

DOCUMENT-IDENTIFIER: US 5492821 A
TITLE: Stabilized polyacrylic saccharide protein conjugates

BSPR:
The linker group includes all of the atoms between the polymer main chain (which is made of the atoms bonded to each other to form the length and longitudinal axis of the polymer) and the amino acid residue of the protein attached to the linker and will separate the main chain of the polymer from the protein by two or more carbon atoms and include at least three hydroxyl groups. In a very important aspect of the invention the linker group will be a saccharide group which includes the residue of a monosaccharide, a disaccharide and a trisaccharide.

DEPR:
Enzymes, antibodies, and the like are complex proteins each with a specific sequence of amino acids. The structure of the protein is critical to the activity of the protein such as the catalytic activity of an enzyme and the capacity of an antibody to recognize ligands.

DEPR:
"Protein" means proteins, including proteins modified to include additional amino groups such as lysine groups, polypeptides, enzymes, antibodies, and the like, which are composed of a specific sequence of amino acids.

DEPR:
The linker group includes all atoms between the polymer main chain and the amino acid residue of the protein attached to the linker group and will separate the main chain of the polymer from the protein by two or more carbon atoms and have at least three hydroxyl groups. As used herein, a linker is a precursor to a linker group. In a very important aspect of the invention the linker group is a saccharide group which includes the residue of a monosaccharide, a disaccharide and a trisaccharide. A linker group which includes hydroxyl groups stabilizes the protein; and while there is not necessarily a defined limited as to the exact number of carbon atoms in a linker group, steric and kinetic considerations limit the size of the linker group to a total of about 60 carbon atoms.

DEPR:
After a linker is coupled with a protein to form a linker group, the availability of an abundant number of hydroxyl groups on the linker group such as on a saccharide linker group provides enhancement of hydrophilic amino acid residue contacts.

DEPR:
Enzymes which can be part of the polymer protein compounds of the invention include enzymes related to the production of fructose, such as glucose isomerase, which operates at 60.degree. to 65.degree. C., hydrolysis of starch by .alpha.-amylase, which occurs at 85.degree. to 110.degree. C. and resolution of D,L-amino acids by amino acetolase at 50.degree. C. Other enzymes which can be a part of the polymer protein compounds of the invention are nucleases, including endonucleases.

DEPR:

Enzymes which can be stabilized as described here include oxidoreductases, transferases, dehydrogenases, transaminases, peptidases, hydrolases, isomerases, mutases and the like. In addition to enzymes already described, other enzymes which could be stabilized herein include but are not limited to malate dehydrogenase; creatine kinase (CK, CPK); alkaline phosphatase (AP, ALP, ALK-phos); aspartate aminotransferase (AST, OT, GOT); alanine aminotransferase (ALT, PT, SGPT); gamma glutamyl transpeptidase (gammaGT, gammaGTP); glutamic oxalacetic transaminase (SGOT); glutamic pyruvic transaminase (GTP); alpha amylase; beta amylase; lactate dehydrogenase (LD, LDH, lactic dehydrogenase); glucose-6-phosphate dehydrogenase (G6PDH); hexokinase (HK); glucose dehydrogenase; glucose oxidase; peroxidase (HRP, HPO, HRPO, PO); glycerol dehydrogenase; glutamate dehydrogenase, cholesterol oxidase; cholesterol esterase; lipase; uricase; urease; glycerol kinase; aldolases; synthetases; nucleases; polymerases; and the like.

DEPR:
The polymer herein can also be used to synthesize peptide bonds. Typically, equimolar solutions containing modified amino acid(s) are dissolved in a solvent system containing an organic solvent such as dioxane or THF (tetrahydrofuran), or a tertiary amine such as (iPr).sub.2 NEt, to form an amino acid mixture. More preferably, the organic solvent is acetonitrile. The solutions dissolved in the solvent system are defined herein as amino acid acceptor and amino acid donor solutions. Examples of such solutions can be found in Table 2 of Example 10.

DEPR:
A conjugated enzyme such as protease (the enzyme incorporated into one of the compounds of the invention) may be added to the amino acid mixture in an amount of about 10.sup.-6 to about 10.sup.-1 mole fraction relative to the amount of amino acid. The conjugated enzyme can be prepared as in Example 8. The reaction is performed at from about 0.degree. to 70.degree. C., at about 1 to about 24 hours. The enzyme polymer conjugate is removed from the formed peptide by filtering and the solvent is evaporated to obtain the resulting peptide.

DETL:
TABLE 2

Enzyme (Solvent, Time)	Acceptor Amino Acid	Donor Amino Acid	Isolated (HPLC) Yield at 23.degree. C. Product (%)
CPC-CT	AcPhe--OEt	Ala--NH.sub.2	AcPheAla--NH.sub.2 97 (100) (Dioxane, 12 h)
CPC-CT*	AcPhe--OEt	Ala--NH.sub.2	AcPheAla--NH.sub.2 94 (98) (THF, 12 h)
CPC-BPN'	CbzLeuLeu--OMe	Leu--O.sup.t Bu	CbzLeuLeuLeu--O.sup.t Bu 68 (95) (Acetonitrile, 22 h)
CPC-BPN'	CbzLeuLeu--OMe	PheLeu--O.sup.t Bu	CbzLeuLeuPheLeu--O.sup.t Bu 92 (98) (Acetronitrile, 24 h)
CPC-BPN'	CbzValLeu--OMe	PheLeu--O.sup.t Bu	CbzValLeuPheLeu--O.sup.t Bu 95 (98) (Acetronitrile, 24 h)
CPC-BPN'	CbzValLeu--OMe	Ala--NH.sub.2	CbzValLeuAla--NH.sub.2 65 (95) (Acetronitrile, 24 h)
CPC-BPN'	CbzLeuLeu--OMe	Ala--NH.sub.2	CbzLeuLeuAla--NH.sub.2 55 (90) (Acetronitrile, 24 h)
CPC-T	CbzPhe--OH	Leu--OMe	CbzPheLeu--OMe 65 (95) (Acetronitrile, 24 h)

*at 50.degree. C.

ORPL:
Margolin et al., Incorporation of D-Amino Acids into Peptides via Enzymatic Condensation in Organic Solvents, 109 J. Am. Chem. Soc. 7885-7887 (1987).

ORPL:
West & Wong, Enzyme-Catalyzed Synthesis of Peptides Containing D-Amino Acids, J. Chem. Soc., Chem. Commun. 417-418 (1986).

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L15: Entry 4 of 10 File: USPT Jun 17, 1997

DOCUMENT-IDENTIFIER: US 5639633 A
TITLE: Method for synthesizing peptides with saccharide linked enzyme polymer conjugates

BSPR:
The linker group includes all of the atoms between the polymer main chain (which is made of the atoms bonded to each other to form the length and longitudinal axis of the polymer) and the amino acid residue of the protein attached to the linker and will separate the main chain of the polymer from the protein by two or more carbon atoms and include at least three hydroxyl groups. In a very important aspect of the invention the linker group will be a saccharide group which includes the residue of a monosaccharide, a disaccharide and a trisaccharide.

DEPR:
Enzymes, antibodies, and the like are complex proteins each with a specific sequence of amino acids. The structure of the protein is critical to the activity of the protein such as the catalytic activity of an enzyme and the capacity of an antibody to recognize ligands.

DEPR:
"Protein" means proteins, including proteins modified to include additional amino groups such as lysine groups, polypeptides, enzymes, antibodies, and the like, which are composed of a specific sequence of amino acids.

DEPR:
The linker group includes all atoms between the polymer main chain and the amino acid residue of the protein attached to the linker group and will separate the main chain of the polymer from the protein by two or more carbon atoms and have at least three hydroxyl groups. As used herein, a linker is a precursor to a linker group. In a very important aspect of the invention the linker group is a saccharide group which includes the residue of a monosaccharide, a disaccharide and a trisaccharide. A linker group which includes hydroxyl groups stabilizes the protein; and while there is not necessarily a defined limited as to the exact number of carbon atoms in a linker group, steric and kinetic considerations limit the size of the linker group to a total of about 60 carbon atoms.

DEPR:
After a linker is coupled with a protein to form a linker group, the availability of an abundant number of hydroxyl groups on the linker group such as on a saccharide linker group provides enhancement of hydrophilic amino acid residue contacts.

DEPR:
Enzymes which can be part of the polymer protein compounds of the invention include enzymes related to the production of fructose, such as glucose isomerase, which operates at 60.degree. to 65.degree. C., hydrolysis of starch by .alpha.-amylase, which occurs at 85.degree. to 110.degree. C. and resolution of D,L-amino acids by amino acetolase at 50.degree. C. Other enzymes which can be a part of the polymer protein compounds of the invention are nucleases, including endonucleases.

DEPR:
Enzymes which can be stabilized as described here include oxidoreductases, transferases, dehydrogenases, transaminases, peptidases, hydrolases, isomerases, mutases and the like. In addition to enzymes already described, other enzymes which could be stabilized herein include but are not limited to malate dehydrogenase; creatine kinase (CK, CPK); alkaline phosphatase (AP, ALP, ALK-phos); aspartate aminotransferase (AST, OT, GOT); alanine aminotransferase (ALT, PT, SGPT); gamma glutamyl transpeptidase (gammaGT, gammaGTP); glutamic oxalacetic transaminase (SGOT); glutamic pyruvic transaminase (GTP); alpha amylase; beta amylase; lactate dehydrogenase (LD, LDH, lactic dehydrogenase); glucose-6-phosphate dehydrogenase (G6PDH); hexokinase (HK); glucose dehydrogenase; glucose oxidase; peroxidase (HRP, HPO, HRPO, PO); glycerol dehydrogenase; glutamate dehydrogenase, cholesterol oxidase; cholesterol esterase; lipase; uricase; urease; glycerol kinase; aldolases; synthetases; nucleases; polymerases; and the like.

DEPR:
The polymer herein can also be used to synthesize peptide bonds. Typically, equimolar solutions containing modified amino acid(s) are dissolved in a solvent system containing an organic solvent such as dioxane or THF (tetrahydrofuran), or a tertiary amine such as (iPr).sub.2 NEt, to form an amino acid mixture. More preferably, the organic solvent is acetonitrile. The solutions dissolved in the solvent system are defined herein as amino acid acceptor and amino acid donor solutions. Examples of such solutions can be found in Table 2 of Example 10.

DEPR:
A conjugated enzyme such as protease (the enzyme incorporated into one of the compounds of the invention) may be added to the amino acid mixture in an amount of about 10.sup.-6 to about 10.sup.-1 mole fraction relative to the amount of amino acid. The conjugated enzyme can be prepared as in Example 8. The reaction is performed at from about 0.degree. to 70.degree. C., at about 1 to about 24 hours. The enzyme polymer conjugate is removed from the formed peptide by filtering and the solvent is evaporated to obtain the resulting peptide.

DETL:
TABLE 2

Enzyme (Solvent, Time) Acceptor Donor Isolated (HPLC) at 23.degree. C. Amino Acid Amino Acid Product Yield (%)

CPC-CT AcPhe-OEt Ala-NH.sub.2 AcPheAla-NH.sub.2 97 (100) (Dioxane, 12 h) CPC-CT* AcPhe-OEt Ala-NH.sub.2 AcPheAla-NH.sub.2 94 (98) (THF, 12h) CPC-BPN' CbzLeuLeu-OMe Leu-O.sup.t Bu CbzLeuLeuLeu-O.sup.t Bu 68 (95) (Acetonitrile, 22 h) CPC-BPN' CbzLeuLeu-OMe PheLeu-O.sup.t Bu CbzLeuLeuPheLeu-O.sup.t Bu 92 (98) (Acetronitrile, 24 h) CPC-BPN' CbzValLeu-OMe PheLeu-O.sup.t Bu CbzValLeuPheLeu-O.sup.t Bu 95 (98) (Acetronitrile, 24 h) CPC-BPN' CbzValLeu-OMe Ala-NH.sub.2 CbzValLeuAla-NH.sub.2 65 (95) (Acetronitrile, 24 h) CPC-BPN' CbzLeuLeu-OMe Ala-NH.sub.2 CbzLeuLeuAla-NH.sub.2 55 (90) (Acetronitrile, 24 h) CPC-T CbzPhe-OH Leu-OMe CbzPheLeu-OMe 65 (95) (Acetronitrile, 24 h)

*at 50.degree. C.

CLPR:
1. A method for making a peptide, the method comprising reacting at least two amino acids with a water soluble enzyme polymer conjugate to produce a peptide and recovering the peptide, the enzyme polymer conjugate comprising covalently bonded enzyme to a polymer, wherein the polymer is selected from the group consisting of a polyvinyl polymer, a polyester polymer, a polyamide polymer and mixtures thereof, the polymer having a main chain molecular weight of at least 5,000, and wherein three or more linker groups covalently bond both the enzyme and polymer and said linking groups have at least three hydroxyl groups and not more than 60 carbon atoms, each of said linker groups being selected

from the group consisting of ##STR26## wherein E=--OH or --SH;

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L15: Entry 2 of 10 File: USPT Apr 7, 1998

DOCUMENT-IDENTIFIER: US 5736625 A
TITLE: Method for stabilizing proteins with saccharide linked protein polymer conjugates

BSPR:
The linker group includes all of the atoms between the polymer main chain (which is made of the atoms bonded to each other to form the length and longitudinal axis of the polymer) and the amino acid residue of the protein attached to the linker and will separate the main chain of the polymer from the protein by two or more carbon atoms and include at least three hydroxyl groups. In a very important aspect of the invention the linker group will be a saccharide group which includes the residue of a monosaccharide, a disaccharide and a trisaccharide.

DEPR:
Enzymes, antibodies, and the like are complex proteins each with a specific sequence of amino acids. The structure of the protein is critical to the activity of the protein such as the catalytic activity of an enzyme and the capacity of an antibody to recognize ligands.

DEPR:
"Protein" means proteins, including proteins modified to include additional amino groups such as lysine groups, polypeptides, enzymes, antibodies, and the like, which are composed of a specific sequence of amino acids.

DEPR:
The linker group includes all atoms between the polymer main chain and the amino acid residue of the protein attached to the linker group and will separate the main chain of the polymer from the protein by two or more carbon atoms and have at least three hydroxyl groups. As used herein, a linker is a precursor to a linker group. In a very important aspect of the invention the linker group is a saccharide group which includes the residue of a monosaccharide, a disaccharide and a trisaccharide. A linker group which includes hydroxyl groups stabilizes the protein; and while there is not necessarily a defined limited as to the exact number of carbon atoms in a linker group, steric and kinetic considerations limit the size of the linker group to a total of about 60 carbon atoms.

DEPR:
After a linker is coupled with a protein to form a linker group, the availability of an abundant number of hydroxyl groups on the linker group such as on a saccharide linker group provides enhancement of hydrophilic amino acid residue contacts.

DEPR:
Enzymes which can be part of the polymer protein compounds of the invention include enzymes related to the production of fructose, such as glucose isomerase, which operates at 60.degree. to 65.degree. C., hydrolysis of starch by .alpha.-amylase, which occurs at 85.degree. to 110.degree. C. and resolution of D,L-amino acids by amino acetolase at 50.degree. C. Other enzymes which can be a part of the polymer protein compounds of the invention are nucleases, including endonucleases.

DEPR:
Enzymes which can be stabilized as described here include oxidoreductases, transferases, dehydrogenases, transaminases, peptidases, hydrolaser, isomerases, mutases and the like. In addition to enzymes already described, other enzymes which could be stabilized herein include but are not limited to malate dehydrogenase; creatine kinase (CK, CPK); alkaline phosphatase (AP, ALP, ALK-phos); aspartate aminotransferase (AST, OT, GOT); alanine aminotransferase (ALT, PT, SGPT); gamma glutamyl transpeptidase (gammaGT, gammaGTP); glutamic oxalacetic transaminase (SGOT); glutamic pyruvic transaminase (GTP); alpha amylase; beta amylase; lactate dehydrogenase (LD, LDH, lactic dehydrogenase); glucose-6-phosphate dehydrogenase (G6PDH); hexokinase (HK); glucose dehydrogenase; glucose oxidase; peroxidase (HRP, HPO, HRPO, PO); glycerol dehydrogenase; glutamate dehydrogenase, cholesterol oxidase; cholesterol esterase; lipase; uricase; urease; glycerol kinase; aldolases; synthetases; nucleases; polymerases; and the like.

DEPR:
The polymer herein can also be used to synthesize peptide bonds. Typically, equimolar solutions containing modified amino acid(s) are dissolved in a solvent system containing an organic solvent such as dioxane or THF (tetrahydrofuran), or a tertiary amine such as (iPr).sub.2 NEt, to form an amino acid mixture. More preferably, the organic solvent is acetonitrile. The solutions dissolved in the solvent system are defined herein as amino acid acceptor and amino acid donor solutions. Examples of such solutions can be found in Table 2 of Example 10.

DEPR:
A conjugated enzyme such as protease (the enzyme incorporated into one of the compounds of the invention) may be added to the amino acid mixture in an amount of about 10.sup.-6 to about 10.sup.-1 mole fraction relative to the amount of amino acid. The conjugated enzyme can be prepared as in Example 8. The reaction is performed at from about 0.degree. to 70.degree. C., at about 1 to about 24 hours. The enzyme polymer conjugate is removed from the formed peptide by filtering and the solvent is evaporated to obtain the resulting peptide.

DETL:
TABLE 2

Enzyme (Solvent, Time)	Acceptor	Donor	Isolated (HPLC) at 23.degree. C.	Amino Acid	Amino Acid	Product	Yield (%)
CPC-CT	AcPhe-OEt	Ala-NH.sub.2	AcPheAla-NH.sub.2	97(100)	(Dioxane, 12 h)		
CPC-CT *	AcPhe-OEt	Ala-NH.sub.2	AcPheAla-NH2	94(98)	(THF, 12 h)		
CPC-BPN'	CbzLeuLeu-OMe	Leu-O.sup.t Bu	CbzLeuLeuLeu-O.sup.t Bu	68(95)	(Acetonitrile, 22 h)		
CPC-BPN'	CbzLeuLeu-OMe	PheLeu-O.sup.t Bu	CbzLeuLeuPheLeu-O.sup.t Bu	92(98)	(Acetronitrile, 24 h)		
CPC-BPN'	CbzValLeu-OMe	PheLeu-O.sup.t Bu	CbzValLeuPheLeu-O.sup.t Bu	95(98)	(Acetronitrile, 24 h)		
CPC-BPN'	CbzValLeu-OMe	Ala-NH.sub.2	CbzValLeuAla-NH.sub.2	65(95)	(Acetronitrile, 24 h)		
CPC-BPN'	CbzLeuLeu-OMe	Ala-NH.sub.2	CbzLeuLeuAla-NH.sub.2	55(90)	(Acetronitrile, 24 h)		
CPC-T	CbzPhe-OH	Leu-OMe	CbzPheLeu-OMe	65(95)	(Acetronitrile, 24 h)		

* at 50.degree. C.

L8: Entry 51 of 71 File: USPT Sep 8, 1998

DOCUMENT-IDENTIFIER: US 5804402 A
TITLE: Reagent

Abstract Text (1):
This invention relates to a reagent for enzymatic determination of an analyte concentration in a patient wherein the degree of oxidation of a coenzyme is measured, characterized in that said reagent is stabilized against oxidation by a coenzyme reduction system comprising an enzyme and substrate pair selected so as to enable continuous regeneration of said coenzyme throughout storage of said reagent. Also disclosed is an improvement in an enzymatic method of determination of an analyte concentration in a sample body fluid wherein the degree of oxidation of a coenzyme is measured, the improvement comprising stabilizing a reagent comprising said coenzyme against oxidation by a coenzyme reduction system comprising an enzyme and substrate pair selected so as to enable continuous regeneration of said coenzyme throughout storage of said reagent. Also disclosed are reagents for the determination of aspartate aminotransferase, alanine aminotransferase, ammonia and urea.

Brief Summary Text (7):
The indirect quantification of enzymes, in particular, the transaminases, aspartate aminotransferase and alanine aminotransferase in sample body fluids may involve contrasting a sample "blank" against a sample in which the enzymatic conversion of an analyte associated with the enzyme of interest has taken place.

Brief Summary Paragraph Table (3):

Substrate	Relative Activity
L-valine	74%
L-isoleucine	58%
L-norvaline	41%
L-norleucine	10%
L-methionine	0.6%
L-cysteine	0.3%

L8: Entry 50 of 71 File: USPT Sep 29, 1998

DOCUMENT-IDENTIFIER: US 5814473 A
**** See image for Certificate of Correction ****
TITLE: Transaminases and aminotransferases

Brief Summary Text (5):
The measurement of alanine aminotransferase and aspartate aminotransferase levels in blood serum is an important diagnostic procedure in medicine, used as an indicator of heart damage and to monitor recovery from the damage.

Detailed Description Text (68):
Transaminases are highly stereoselective, and most use L-amino acids as substrates. Using the approach disclosed in a commonly assigned, copending provisional application Ser. No. 60/008,316, filed on Dec. 7, 1995 and entitled "Combinatorial Enzyme Development," the disclosure of which is incorporated herein by reference in its entirety, one can convert the transaminases of the invention to use D-amino acids as substrates. Such conversion makes possible a broader array of transaminase applications. For instance, D-valine can be used in the manufacture of synthetic pyrethroids. D-phenylglycine and its derivatives can be useful as components of .beta.-lactam antibiotics. Further, the thermostable transaminases have superior stability at higher temperatures and in organic solvents. Thus, they are better suited to utilize either L- and/or D-amino acids for production of optically pure chiral compounds used in pharmaceutical, agricultural, and other chemical manufactures.

L8: Entry 44 of 71 File: USPT Oct 5, 1999

DOCUMENT-IDENTIFIER: US 5962283 A
TITLE: Transminases and amnotransferases

Brief Summary Text (5):
The measurement of alanine aminotransferase and aspartate aminotransferase levels in blood serum is an important diagnostic procedure in medicine, used as an indicator of heart damage and to monitor recovery from the damage.

Brief Summary Text (81):
Transaminases are highly stereoselective, and most use L-amino acids as substrates. Using the approach disclosed in a commonly assigned, copending provisional application Ser. No. 60/008,316, filed on Dec. 7, 1995 and entitled "Combinatorial Enzyme Development," the disclosure of which is incorporated herein by reference in its entirety, one can convert the transaminases of the invention to use D-amino acids as substrates. Such conversion makes possible a broader array of transaminase applications. For instance, D-valine can be used in the manufacture of synthetic pyrethroids. D-phenylglycine and its derivatives can be useful as components of .beta.-lactam antibiotics. Further, the thermostable transaminases have superior stability at higher temperatures and in organic solvents. Thus, they are better suited to utilize either L- and/or D-amino acids for production of optically pure chiral compounds used in pharmaceutical, agricultural, and other chemical manufactures.

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Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: 114 and 113

Display: 10 **Documents in Display Format:** CIT **Starting with Number** 1

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Search History

Today's Date: 4/26/2001

DB Name	Query	Hit Count	Set Name
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l14 and l13	10	L15
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l2 or (amino acid)	145677	L14
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l5 same l1	24	L13
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l5 and l1	390	L12
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l9 not l10	8	L11
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l5 and l9	11	L10
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l7 same l2	19	L9
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l7 and l5	645	L8
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	aminotransferase	1166	L7
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l1 same l2	5	L6
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	stabiliz$5 or stabl$5	1264710	L5
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	stabiliz$5	505158	L4
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l1 and l2	108	L3
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	valine or proline	27431	L2
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	(aspartate aminotransferase) or (alanine aminotransferase)	674	L1

Trying 3106016892...Open

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* * * * * * * * * * Welcome to STN International * * * * * * * * * *

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NEWS 3	Feb 06	Engineering Information Encompass files have new names
NEWS 4	Feb 16	TOXLINE no longer being updated
NEWS 5	Apr 23	Search Derwent WPINDEX by chemical structure
NEWS 6	Apr 23	PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA

NEWS EXPRESS	April 18 CURRENT WINDOWS VERSION IS V6.0, CURRENT MACINTOSH VERSION IS V5.0C (ENG) AND V5.0JB (JP), AND CURRENT DISCOVER FILE IS DATED 04/06
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FILE 'HOME' ENTERED AT 12:20:46 ON 26 APR 2001

=> index bioscience
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED

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FULL ESTIMATED COST	0.15	0.15

INDEX 'ADISALERTS, ADISINSIGHT, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, DRUGNL, ...' ENTERED AT 12:21:11 ON 26 APR 2001

59 FILES IN THE FILE LIST IN STNINDEX

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=> file reg

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.45	0.60

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DICTIONARY FILE UPDATES: 25 APR 2001 HIGHEST RN 332836-91-6

TSCA INFORMATION NOW CURRENT THROUGH January 11, 2001

Please note that search-term pricing does apply when conducting SmartSELECT searches.

Structure search limits have been increased. See HELP SLIMIT for details.

=> s aspartate aminotransferase/cn
L1 1 ASPARTATE AMINOTRANSFERASE/CN

=> d

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2001 ACS
RN 9000-97-9 REGISTRY
CN Aminotransferase, aspartate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 2-Oxoglutarate-glutamate aminotransferase
CN Aspartate .alpha.-ketoglutarate transaminase
CN **Aspartate aminotransferase**
CN Aspartate-2-oxoglutarate transaminase
CN Aspartic acid aminotransferase
CN Aspartic aminotransferase
CN Aspartyl aminotransferase
CN AST
CN E.C. 2.6.1.1
CN Glutamate 2-oxoglutarate transaminase
CN Glutamate-oxalacetate aminotransferase
CN Glutamate-oxalate transaminase
CN Glutamate-oxaloacetate transaminase
CN Glutamic-aspartic aminotransferase
CN Glutamic-aspartic transaminase
CN Glutamic-oxalacetic transaminase
CN Glutamic-oxalic transaminase
CN GOT
CN GOT (enzyme)
CN L-Aspartate aminotransferase
CN L-Aspartate transaminase
CN L-Aspartate-.alpha.-ketoglutarate transaminase
CN L-Aspartate-2-ketoglutarate aminotransferase
CN L-Aspartate-2-oxoglutarate aminotransferase
CN L-Aspartate-2-oxoglutarate-transaminase
CN L-Aspartic aminotransferase
CN Oxalacetate-aspartate aminotransferase
CN Oxaloacetate transferase
DR 9013-64-3, 9014-29-3, 9016-19-7, 9036-26-4, 9061-83-0, 61461-53-8, 139074-52-5
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, CASREACT, CHEMCATS, CHEMLIST, CIN, CSCHEM, EMBASE, IFICDB,

IFIPAT, IFIUDB, IPA, MEDLINE, MSDS-OHS, NAPRALERT, NIOSHTIC, PROMT,
TOXLINE, TOXLIT, USPATFULL
Other Sources: EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
14782 REFERENCES IN FILE CA (1967 TO DATE)
85 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
14805 REFERENCES IN FILE CAPLUS (1967 TO DATE)

=> s alanine aminotransferase/cn
L2 1 ALANINE AMINOTRANSFERASE/CN

=> d

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2001 ACS
RN 9000-86-6 REGISTRY
CN Aminotransferase, alanine (8CI, 9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Alanine aminotransferase**
CN Alanine transaminase
CN Alanine-.alpha.-ketoglutarate aminotransferase
CN Alanine-oxaloacetate aminotransferase
CN Alanine-pyruvate aminotransferase
CN ALT
CN E.C. 2.6.1.2
CN Glutamic acid-pyruvic acid transaminase
CN Glutamic-alanine transaminase
CN Glutamic-pyruvic aminotransferase
CN Glutamic-pyruvic transaminase
CN GPT
CN L-Alanine aminotransferase
CN L-Alanine transaminase
CN L-Alanine-.alpha.-ketoglutarate aminotransferase
CN Pyruvate transaminase
CN Pyruvate-alanine aminotransferase
CN Pyruvate-glutamate transaminase
CN SGPT
DR 9013-65-4, 9014-30-6, 9048-64-0, 139074-53-6
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA,
CAPLUS, CASREACT, CHEMCATS, CHEMLIST, CIN, CSCHEM, CSNB, EMBASE,
IFICDB,
IFIPAT, IFIUDB, MEDLINE, MSDS-OHS, NAPRALERT, NIOSHTIC, PROMT, TOXLINE,
TOXLIT, USPATFULL
Other Sources: EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
10974 REFERENCES IN FILE CA (1967 TO DATE)
14 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
10995 REFERENCES IN FILE CAPLUS (1967 TO DATE)

=> index bioscience
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION

FULL ESTIMATED COST 11.22 11.82

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59 FILES IN THE FILE LIST IN STNINDEX

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=> s l1 or l2

0*	FILE ADISALERTS
1000	FILE AGRICOLA
140	FILE ANABSTR
0*	FILE AQUASCI
304	FILE BIOBUSINESS
0*	FILE BIOCOMMERCE
7779	FILE BIOSIS
2676	FILE BIOTECHNO
0*	FILE CABA
0*	FILE CAPLUS
0*	FILE CEABA-VTB
8	FILE CIN
0*	FILE CONFSCI
0*	FILE CROPB
0*	FILE CROPU
0*	FILE DDFB
0*	FILE DDFU
0*	FILE DGENE
0*	FILE DRUGB
0*	FILE DRUGU
0*	FILE EMBAL
0*	FILE ESBIOBASE
0*	FILE FOMAD
0*	FILE FOREGE
0*	FILE FROSTI
0*	FILE GENBANK
0*	FILE HEALSAFE
0*	FILE IFIPAT
0*	FILE KOSMET
0*	FILE LIFESCI
0*	FILE MEDICONF
1264	FILE MEDLINE

45 FILES SEARCHED...

0*	FILE NTIS
0*	FILE OCEAN
0*	FILE PASCAL
0*	FILE PHIC
0*	FILE PHIN
119	FILE PROMT
0*	FILE SCISEARCH
0*	FILE USPATFULL

8 FILES HAVE ONE OR MORE ANSWERS, 59 FILES SEARCHED IN STNINDEX

L3 QUE L1 OR L2

```
=> s valine? or proline?
        102   FILE ADISALERTS
         62   FILE ADISINSIGHT
       3361   FILE AGRICOLA
        988   FILE ANABSTR
        923   FILE AQUASCI
       1357   FILE BIOBUSINESS
         47   FILE BIOCOMMERCE
      39518   FILE BIOSIS
       2172   FILE BIOTECHABS
       2172   FILE BIOTECHDS
      11612   FILE BIOTECHNO
      10774   FILE CABA
       4033   FILE CANCERLIT
      78892   FILE CAPLUS
        453   FILE CEABA-VTB
         55   FILE CEN
         47   FILE CIN
        483   FILE CONFSCI
        149   FILE CROPB
        393   FILE CROPU
       2061   FILE DDFB
       1688   FILE DDFU
       3445   FILE DGENE
       2061   FILE DRUGB
         12   FILE DRUGLAUNCH
        984   FILE DRUGMONOG2
         35   FILE DRUGNL
       2480   FILE DRUGU
         59   FILE DRUGUPDATES
        200   FILE EMBAL
      27954   FILE EMBASE
       7795   FILE ESBIOBASE
          1   FILE FOMAD
         47   FILE FOREGE
        761   FILE FROSTI
35 FILES SEARCHED...
       2685   FILE FSTA
       5967   FILE GENBANK
         47   FILE HEALSAFE
       3325   FILE IFIPAT
       1975   FILE JICST-EPLUS
         48   FILE KOSMET
       9234   FILE LIFESCI
      35095   FILE MEDLINE
        331   FILE NIOSHTIC
        304   FILE NTIS
        253   FILE OCEAN
       9871   FILE PASCAL
        112   FILE PHAR
          1   FILE PHIC
         49   FILE PHIN
       1646   FILE PROMT
      22030   FILE SCISEARCH
        117   FILE SYNTHLINE
       6734   FILE TOXLINE
       9935   FILE TOXLIT
      20991   FILE USPATFULL
       4548   FILE WPIDS
       4548   FILE WPINDEX
```

58 FILES HAVE ONE OR MORE ANSWERS, 59 FILES SEARCHED IN STNINDEX

L4 QUE VALINE? OR PROLINE?

=> s (aspartate aminotransferase?) or (alanine aminotransferase?)

1067	FILE ADISALERTS
39	FILE ADISINSIGHT
1059	FILE AGRICOLA
261	FILE ANABSTR
279	FILE AQUASCI
316	FILE BIOBUSINESS
23	FILE BIOCOMMERCE
12011	FILE BIOSIS
113	FILE BIOTECHABS
113	FILE BIOTECHDS
3675	FILE BIOTECHNO
5911	FILE CABA
1495	FILE CANCERLIT
12794	FILE CAPLUS
39	FILE CEABA-VTB
8	FILE CIN
116	FILE CONFSCI
19	FILE CROPB
321	FILE CROPU
164	FILE DDFB
2190	FILE DDFU
861	FILE DGENE
164	FILE DRUGB
10	FILE DRUGNL
2974	FILE DRUGU
13	FILE DRUGUPDATES
79	FILE EMBAL
20823	FILE EMBASE
2182	FILE ESBIOBASE

32 FILES SEARCHED...

58	FILE FROSTI
97	FILE FSTA
1728	FILE GENBANK
98	FILE HEALSAFE
52	FILE IFIPAT
566	FILE JICST-EPLUS
6	FILE KOSMET
2184	FILE LIFESCI
9483	FILE MEDLINE
546	FILE NIOSHTIC
93	FILE NTIS
72	FILE OCEAN
3478	FILE PASCAL
7	FILE PHAR
61	FILE PHIN
412	FILE PROMT
7042	FILE SCISEARCH
3734	FILE TOXLINE
3522	FILE TOXLIT
531	FILE USPATFULL
101	FILE WPIDS
101	FILE WPINDEX

51 FILES HAVE ONE OR MORE ANSWERS, 59 FILES SEARCHED IN STNINDEX

Q

N

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 NEWS  4  Feb 16   TOXLINE no longer being updated
 NEWS  5  Apr 23   Search Derwent WPINDEX by chemical structure
 NEWS  6  Apr 23   PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA

 NEWS EXPRESS  April 18 CURRENT WINDOWS VERSION IS V6.0,
               CURRENT MACINTOSH VERSION IS V5.0C (ENG) AND V5.0JB (JP),
               AND CURRENT DISCOVER FILE IS DATED 04/06
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FILE 'HOME' ENTERED AT 12:56:36 ON 26 APR 2001

=> file ca, biosis, medline
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                        ENTRY      SESSION
FULL ESTIMATED COST                                      0.15         0.15

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FILE 'BIOSIS' ENTERED AT 12:57:06 ON 26 APR 2001
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FILE 'MEDLINE' ENTERED AT 12:57:06 ON 26 APR 2001

=> s (aspartate aminotransferase?) or (alanine aminotransferase?)
L1        34077 (ASPARTATE AMINOTRANSFERASE?) OR (ALANINE AMINOTRANSFERASE?)

=> s valine or proline
L2       151467 VALINE OR PROLINE
```

```
=> s l1 (p) l2
L3          238 L1 (P) L2

=> s stab?
L4      1677944 STAB?

=> s l3 and l4
L5           16 L3 AND L4

=> dup re, l5
'RE' IS NOT VALID HERE
Enter "REMOVE" to identify and remove duplicate answers.
Enter "IDENTIFY" to identify duplicate answers in the answer set.
Enter "ONLY" to identify and create an answer set containing only
duplicate records.
ENTER REMOVE, IDENTIFY, ONLY, OR (?):dup rem l5
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duplicate records.
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Enter "REMOVE" to identify and remove duplicate answers.
Enter "IDENTIFY" to identify duplicate answers in the answer set.
Enter "ONLY" to identify and create an answer set containing only
duplicate records.
ENTER REMOVE, IDENTIFY, ONLY, OR (?):d 1-16 ab,bib
'D 1-16 AB,BIB' IS NOT VALID HERE
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Enter "IDENTIFY" to identify duplicate answers in the answer set.
Enter "ONLY" to identify and create an answer set containing only
duplicate records.
ENTER REMOVE, IDENTIFY, ONLY, OR (?):.
Enter "REMOVE" to identify and remove duplicate answers.
Enter "IDENTIFY" to identify duplicate answers in the answer set.
Enter "ONLY" to identify and create an answer set containing only
duplicate records.
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PROCESSING COMPLETED FOR L5
L6           10 DUP REMOVE L5 (6 DUPLICATES REMOVED)

=> d 1-10 ab,bib

L6   ANSWER 1 OF 10  CA  COPYRIGHT 2001 ACS
AB   Aspartate aminotransferase or alanine
     aminotransferase in liq. media such as serum or buffered soln. is
     stabilized by adding valine, proline, or both
     into the media to assure the accuracy of clin. studies.  The enzymes can
     be further stabilized by adding a sol. protein such as albumin
     or gelatin into the media.
AN   131:319672  CA
TI   Stabilization of aminotransferase using amino acids
IN   Baba, Toshiyuki; Tabata, Hiromasa; Nagamatsu, Katashi; Watazu, Yoshifumi;
     Aoki, Ryoji
PA   International Reagents Corporation, Japan; Asahi Chemical Industry Co.,
     Ltd.
SO   PCT Int. Appl., 30 pp.
     CODEN: PIXXD2
DT   Patent
LA   Japanese
```

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9955850	A1	19991104	WO 1999-JP2205	19990423

W: CN, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRAI JP 1998-131159 19980424
RE.CNT 9
RE
(2) Beckman Instruments Inc; EP 16573 A CA
(3) Beckman Instruments Inc; US 4325832 A CA
(4) Beckman Instruments Inc; JP 55-141194 A 1980 CA
(5) Iwan Endore Modorobich; US 4652524 A CA
(6) Iwan Endore Modorobich; EP 49475 A CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 10 CA COPYRIGHT 2001 ACS DUPLICATE 1
AB To elucidate the role of the two conserved cis-**proline** residues of **aspartate aminotransferase** (AspAT), one double and two single mutants of the enzyme from Escherichia coli (EcAspAT) were prepd.: P138A, P195A and P138A/P195A in which the two prolines were replaced by alanine. The crystal structures of P195A and P138A/P195A have been detd. at 2.3-2.1 .ANG. resoln. The wild-type geometry, including the cis conformation of the 194-195 peptide bond is retained upon substitution of **proline** 195 by alanine, whereas the trans conformation is adopted at the 137-138 peptide bond. Quite surprisingly, the replacement of each of the two prolines by alanine does not significantly affect either the activity or the **stability** of the protein. All the three mutants follow the same pathway as the wild type for unfolding equil. induced by guanidine hydrochloride [Herold, M., and Kirschner, K. (1990) Biochem. 29, 1907-1913]. The kinetics of renaturation of P195A, where the alanine retains the wild-type cis conformation, is faster than wild type, whereas renaturation of P138A, which adopts the trans conformation, is slower. We conclude that cis-prolines seem to have been retained throughout the evolution of **aspartate aminotransferase** to possibly play a subtle role in directing the traffic of intermediates toward the unique structure of the native state, rather than to respond to the needs for a specific catalytic or functional role.
AN 130:164805 CA
TI Functional and Structural Analysis of cis-**Proline** Mutants of Escherichia coli **Aspartate Aminotransferase**
AU Birolo, Leila; Malashkevich, Vladimir N.; Capitani, Guido; De Luca, Fabio; Moretta, Alma; Jansonius, Johan N.; Marino, Gennaro
CS Dipartimento di Chimica Organica e Biologica, Universita Federico II, Naples, I-80134, Italy
SO Biochemistry (1999), 38(3), 905-913
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
RE.CNT 45
RE
(1) Birolo, L; Eur J Biochem 1995, V232, P859 CA

(3) Garnier, A; Eur J Biochem 1993, V216, P763 CA
(4) Gloss, L; Biochemistry 1992, V31, P32 CA
(5) Goldberg, J; Biochemistry 1991, V30, P305 CA
(6) Herold, M; Biochemistry 1990, V29, P1907 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 10 CA COPYRIGHT 2001 ACS DUPLICATE 2
AB The adiabatic compressibility (.hivin..beta.s) was detd., by the precise sound velocity and d. measurements, for a series of single amino acid substituted mutant enzymes of Escherichia coli dihydrofolate reductase (DHFR) and **aspartate aminotransferase** (AspAT). Interestingly, the .hivin..beta.s values of both DHFR and AspAT were influenced markedly by the mutations at glycine-121 and **valine** -39, resp., in which the magnitude of the change was proportional to the enzyme activity. This result demonstrates that the local change of the primary structure plays an important role in at. packing and protein dynamics, which leads to the modified **stability** and enzymic function. This is the first report on the compressibility of mutant proteins.
AN 124:224204 CA
TI A large compressibility change of protein induced by a single amino acid substitution
AU Gekko, Kunihiko; Tamura, Youjiro; Ohmae, Eiji; Hayashi, Hideyuki; Kagamiyama, Hiroyuki; Ueno, Hiroshi
CS Fac. Science, Hiroshima Univ., Higashi-Hiroshima, 739, Japan
SO Protein Sci. (1996), 5(3), 542-5
CODEN: PRCIEI; ISSN: 0961-8368
DT Journal
LA English

L6 ANSWER 4 OF 10 CA COPYRIGHT 2001 ACS
AB A notable feature of porcine cytosolic aspartate aminotransferase is the closure of the active site cleft by a mobile amino-terminal segment (residues 15-40) upon binding substrate. The functional roles of Val17 and Phe18, residues that are part of the mobile loop, have been studied in the site-directed mutants in which the size and hydrophobic nature of these residues have been changed. Absorption, CD spectra, susceptibility to protease 401, and thermal **stability** did not differ appreciably between wild type and mutant enzymes. In the overall transamination between aspartate and 2-oxoglutarate, V17A represented a typical Km mutant while V17I retained the substrate binding affinity fairly well. In contrast, replacement of Phe18 by Ala resulted in a large decrease in both catalytic rate and binding affinity for substrates. F18W, F18Y, and F18H showed a moderate decrease in kcat and a considerable increase in Km values. Single-turnover reactions with four individual substrates yielded analogous results to those obtained for the overall reaction and, in addn., revealed that k/Kd values of mutants F18A and F18H were over 10 times lower for C5 substrates (glutamate and 2-oxoglutarate) than those for C4 substrates (aspartate and oxalacetate). All mutant enzymes showed variously increased Kd values for substrate analogs such as 2-methylaspartate, succinate, and glutarate. 1H NMR observations of F18H, in which His18 served as a built-in probe, were in accord with the behavior that would be expected from the conformational transition. The authors conclude that, although Val17 and Phe18 may not be essential for

catalysis, the presence of a bulky residue of appropriate size at each position is crit. for productive binding of substrate.

AN 121:173879 CA

TI Functional role of the amino-terminal mobile segment in catalysis by porcine cytosolic aspartate aminotransferase. Critical importance of Val17 and Phe18 for productive binding of substrates

AU Nishimura, Kosuke; Higaki, Tsuyoshi; Okamura, Hitoshi; Tanase, Sumio

CS Sch. Med., Kumamoto Univ., Kumamoto, 860, Japan

SO J. Biol. Chem. (1994), 269(40), 24712-18
CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

L6 ANSWER 5 OF 10 CA COPYRIGHT 2001 ACS DUPLICATE 3

AB The retina of honeybee drone is a nervous tissue with a crystal-like structure in which glial cells and photoreceptor neurons constitute 2 distinct metabolic compartments. The phosphorylation of glucose and its subsequent incorporation into glycogen occur in glia, whereas O2 consumption (QO2) occurs in the photoreceptors. Exptl. evidence showed that glia phosphorylate glucose and supply the photoreceptors with metabolic substrates. The authors aimed to identify these transferred substrates. Using ion-exchange and reversed-phase HPLC and gas chromatog.-mass spectrometry, the authors demonstrated that >50% of 14C(U)-glucose entering the glia is transformed to alanine by transamination of pyruvate and glutamate. In the absence of extracellular glucose, glycogen is used to make alanine; thus, its pool size in isolated retinas is maintained **stable** or even increased. The authors' model proposes that the formation of alanine occurs in the glia, thereby maintaining the redox potential of this cell and contributing to NH3 homeostasis. Alanine is released into the extracellular space and is then transported into photoreceptors using an Na+-dependent transport system. Purified suspensions of photoreceptors have similar **alanine aminotransferase** activity as glial cells and transform 14C-alanine to glutamate, aspartate, and CO2. Therefore, the alanine entering photoreceptors is transaminated to pyruvate, which in turn enters the Krebs cycle. **Proline** also supplies the Krebs cycle by making glutamate and, in turn, the intermediate .alpha.-ketoglutarate. Light stimulation caused a 200% increase of QO2 and a 50% decrease of **proline** and of glutamate. Also, the prodn. of 14CO2 from 14C-**proline** was increased. The use of these amino acids would sustain about half of the light-induced .DELTA.QO2, the other half being sustained by glycogen via alanine formation. The use of **proline** meets a necessary anaplerotic function in the Krebs cycle, but implies high NH3 prodn. The results showed that alanine formation fixes NH3 at a rate exceeding glutamine formation. This is consistent with the rise of a glial pool of alanine upon photostimulation. In conclusion, the results strongly support a nutritive function for glia.

AN 121:31478 CA

TI Glial cells transform glucose to alanine, which fuels the neurons in the honeybee retina

AU Tsacopoulos, M.; Veuthey, A. L.; Saravelos, S. G.; Perrottet, P.; Tsoupras, G.

CS Sch. Med., Univ. Geneva, Geneva, 1211, Switz.

SO J. Neurosci. (1994), 14(3, Pt. 1), 1339-51
CODEN: JNRSDS; ISSN: 0270-6474

DT Journal
LA English

L6 ANSWER 6 OF 10 MEDLINE
AB The functional roles of Val37 and Gly38 in porcine cytosolic aspartate aminotransferase have been studied in the site-directed mutants V37A, G38A, and G38S where the size and hydrophobic character of these residues has been altered. Previous x-ray studies have shown that Val37 and Gly38, which are part of a flexible loop, interact directly with bound substrate.

From x-ray and solution experiments we find that the V37A, G38A, and G38S mutations do not cause significant perturbations to the unliganded enzyme.

Replacing Val37 with a less bulky alanine residue does not affect the maximal catalytic rate (kcat), but it does increase significantly the Michaelis constants for substrates in the overall transamination reaction between aspartate and 2-oxoglutarate. On the other hand, replacing Gly38 with alanine or serine results in striking decreases in kcat to 5 and 0.6%, respectively, of the value observed for the wild-type enzyme, as well as in considerable increases in Km values. Consequently, the catalytic competence, kcat/Km, decreases by 3 orders of magnitude for G38A

and by 4 orders of magnitude for G38S. Single turnover reactions of G38A and G38S with four individual substrates (aspartate, glutamate, oxalacetate, and 2-oxoglutarate) are characterized by kinetic parameters that are largely consistent with those of the overall reaction. In addition, the mutations at position 38 impair more seriously the catalytic

competence of the enzyme toward C5-substrates than toward C4-substrates. We conclude that Gly38 is probably required for proper function of the enzyme because it permits a high level of flexibility for the 36-39 peptide, which in turn allows the essential substrate-induced movement of the small domain.
AN 94043333 MEDLINE
DN 94043333 PubMed ID: 8227035
TI Functional roles of **valine** 37 and glycine 38 in the mobile loop of porcine cytosolic **aspartate aminotransferase**.
AU Pan Q W; Tanase S; Fukumoto Y; Nagashima F; Rhee S; Rogers P H; Arnone A; Morino Y
CS Department of Biochemistry, Kumamoto University School of Medicine, Japan.
NC GM-40852 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Nov 25) 268 (33) 24758-65. Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199312
ED Entered STN: 19940117
Last Updated on STN: 19980206
Entered Medline: 19931220

L6 ANSWER 7 OF 10 CA COPYRIGHT 2001 ACS
AB Aspartate aminotransferase from E. coli, which had been denatured by guanidinium chloride, refolded and reassembled to active dimers in two distinct phases. The unfolded monomer U collapsed within 20 s to an intermediate I* that was inactive, fluoresced more strongly than, but had the same peptide CD signal as the native dimer. The formation of crosslinkable dimers, as well as the recovery of enzyme activity, occurred

with a biphasic progress curve which was independent of protein concn. The half-lives of the two phases were 100 s and 2000 s. The data are consistent with a three-step mechanism, in which the overall rate of reassembly is detd. by an isomerization of I* to the assembly-competent monomer M. The latter does no accumulate because it dimerizes rapidly to the active enzyme (D). Reassembly of the enzyme from the compact intermediate M*, which is **stable** at 1.0M guanidinium chloride, also proceeded in a rapid and a slow phase. Moreover, the formation of M* from the unfolded state was rapid, whereas its refolding to the native dimer was slow. Both the transient intermediate I* and the equil. intermediate M* qualify as collapsed intermediate or molten globule states.

AN 116:230721 CA
TI Collapsed intermediates in the reconstitution of dimeric aspartate aminotransferase from Escherichia coli
AU Leistler, Bernd; Herold, Marzell; Kirschner, Kasper
CS Dep. Biophys. Chem., Univ. Basel, Basel, CH-4056, Switz.
SO Eur. J. Biochem. (1992), 205(2), 603-11
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L6 ANSWER 8 OF 10 CA COPYRIGHT 2001 ACS
AB The effect of i.m. administration of hydrocortisone (I) [50-23-7] (10 mg/day per animal) for 5 days on the content of the amino acids belonging to the glutamate family in different regions of the mouse brain, along with the activities of glutamine synthetase [9023-70-5], glutamate dehydrogenase [9029-12-3], and **aspartate aminotransferase** [9000-97-9], **alanine aminotransferase** [9000-86-6], tyrosine aminotransferase [9014-55-5] and ornithine [70-26-8] was studied. The activity of **proline** oxidase [9029-17-8] was also studied in these regions. The activities of Na+, K+-ATPase [9000-83-3] together with the content of RNA and protein were also estd. A decrease in the amino acids of the glutamate family in all 3 regions was obsd. with an increase in glutamate dehydrogenase activity in the cerebral cortex. A decrease in the protein content was also obsd., mainly in the brain stem. An increase in Na+, K+-ATPase activity was obsd. in all 3 regions, with the greatest increase occurring in the cerebral cortex. Apparently, I triggers increased utilization of glutamate in brain as an alternative to glucose, thereby shifting N metab. toward catabolism. The increased activity of Na+, K+-ATPase under these conditions would further aggravate the same and may lead to membrane **stabilization**.

AN 88:45451 CA
TI Metabolic effects of hydrocortisone in mouse brain
AU Sadasivudu, B.; Rao, T. Indira; Radha, C.; Murthy, Krishna
CS Dep. Biochem., Kurnool Med. Coll., Kurnool, India
SO Neurochem. Res. (1977), 2(5), 521-32
CODEN: NEREDZ
DT Journal
LA English

L6 ANSWER 9 OF 10 MEDLINE
AN 68130568 MEDLINE
DN 68130568 PubMed ID: 5688916
TI Interaction of rat liver **alanine aminotransferase** with L-**proline**.
AU Segal H L; Abraham G J; Matsuzawa T

SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1968 Jan 11) 30 (1) 63-8.
Journal code: 9Y8; 0372516. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 196804
ED Entered STN: 19900101
Last Updated on STN: 19980206
Entered Medline: 19680410

L6 ANSWER 10 OF 10 CA COPYRIGHT 2001 ACS
AB Expts. were done on **alanine aminotransferase** (I). Rat liver I and I from other organs differed in activity from testis I. Enzyme activity of I was detd. as a function of temp. In the temp. range 55-65.degree., an unexplained break in the curve after .apprx.75% inactivation was regularly observed. First-order decay consts. were obtained in the 1st part of the curve. In protecting liver I against inactivation, arginine, lysine, and ornithine were without effect. Other amino acids were protective, **proline** being the most protective. The prepn. of I from various organs is described. At pH 5.0, 30% (NH4)2SO4 pptd. liver I and also lung, heart, diaphragm, and skeletal muscle I. For pptn. of testis I, 50% concns. of (NH4)2SO4 were required. Antisera were produced in rabbits by injecting liver I. In immunochem. test, the antisera gave a single pptn. band (line of identity) for liver I and for I from diaphragm, skeletal muscle, heart, and lung. Testis I did not react with antisera against liver I. The I from all tissues migrated at the same rate as liver I in acrylamide gel electrophoresis with the exception of testis I, which migrated at .apprx.1/2 the rate. Detns. (in triplicate) of the extinction coeff. of liver I at the absorbancy peak, 278 m.mu., indicated an av. sp. activity of 388 units/mg. protein for liver I and a pyridoxal-P/mole value of 2.0. The results demonstrated that liver I and testis are distinctly different.
AN 70:84593 CA
TI **Stability** of rat liver alanine aminotransferase and forms of the enzyme in other tissues
AU Segal, Harold L.; Abraham, George J.; Schatz, Lillian
CS State Univ. of New York, New York, N. Y., USA
SO Symp. Pyridoxal Enzymes, 3rd (1968), Meeting Date 1967, 37-42.
Editor(s):
Yamada, Kozo. Publisher: Maruzen Co. Ltd., Tokyo, Japan.
CODEN: 20KFAV
DT Conference
LA English

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 NEWS  4  Feb 16   TOXLINE no longer being updated
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 NEWS  6  Apr 23   PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA

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               AND CURRENT DISCOVER FILE IS DATED 04/06
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=> s (ASPARTATE AMINOTRANSFERASE?) OR (ALANINE AMINOTRANSFERASE?)
L1        34077 (ASPARTATE AMINOTRANSFERASE?) OR (ALANINE AMINOTRANSFERASE?)

=> s valine
L2        65762 VALINE
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=> s l1 (p) l2
L3          139 L1 (P) L2

=> s stab?
L4      1677944 STAB?

=> s l3 and l4
L5            6 L3 AND L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6            4 DUP REM L5 (2 DUPLICATES REMOVED)

=> d 1-4 ab,bib

L6   ANSWER 1 OF 4  CA  COPYRIGHT 2001 ACS
AB   Aspartate aminotransferase or alanine
     aminotransferase in liq. media such as serum or buffered soln. is
     stabilized by adding valine, proline, or both into the
     media to assure the accuracy of clin. studies.  The enzymes can be
further
     stabilized by adding a sol. protein such as albumin or gelatin
     into the media.
AN   131:319672  CA
TI   Stabilization of aminotransferase using amino acids
IN   Baba, Toshiyuki; Tabata, Hiromasa; Nagamatsu, Katashi; Watazu, Yoshifumi;
     Aoki, Ryoji
PA   International Reagents Corporation, Japan; Asahi Chemical Industry Co.,
     Ltd.
SO   PCT Int. Appl., 30 pp.
     CODEN: PIXXD2
DT   Patent
LA   Japanese
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   WO 9955850         A1   19991104       WO 1999-JP2205   19990423
         W:  CN, JP, US
         RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE
PRAI JP 1998-131159          19980424
RE.CNT  9
RE
(2) Beckman Instruments Inc; EP 16573 A CA
(3) Beckman Instruments Inc; US 4325832 A CA
(4) Beckman Instruments Inc; JP 55-141194 A 1980 CA
(5) Iwan Endore Modorobich; US 4652524 A CA
(6) Iwan Endore Modorobich; EP 49475 A CA
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L6   ANSWER 2 OF 4  CA  COPYRIGHT 2001 ACS              DUPLICATE 1
AB   The adiabatic compressibility (.hivin..beta.s) was detd., by the precise
     sound velocity and d. measurements, for a series of single amino acid
     substituted mutant enzymes of Escherichia coli dihydrofolate reductase
     (DHFR) and aspartate aminotransferase (AspAT).
     Interestingly, the .hivin..beta.s values of both DHFR and AspAT were
     influenced markedly by the mutations at glycine-121 and valine
     -39, resp., in which the magnitude of the change was proportional to the
     enzyme activity.  This result demonstrates that the local change of the
     primary structure plays an important role in at. packing and protein
```

dynamics, which leads to the modified **stability** and enzymic function. This is the first report on the compressibility of mutant proteins.

AN 124:224204 CA
TI A large compressibility change of protein induced by a single amino acid substitution
AU Gekko, Kunihiko; Tamura, Youjiro; Ohmae, Eiji; Hayashi, Hideyuki; Kagamiyama, Hiroyuki; Ueno, Hiroshi
CS Fac. Science, Hiroshima Univ., Higashi-Hiroshima, 739, Japan
SO Protein Sci. (1996), 5(3), 542-5
CODEN: PRCIEI; ISSN: 0961-8368
DT Journal
LA English

L6 ANSWER 3 OF 4 CA COPYRIGHT 2001 ACS
AB A notable feature of porcine cytosolic aspartate aminotransferase is the closure of the active site cleft by a mobile amino-terminal segment (residues 15-40) upon binding substrate. The functional roles of Val17 and Phe18, residues that are part of the mobile loop, have been studied in the site-directed mutants in which the size and hydrophobic nature of these residues have been changed. Absorption, CD spectra, susceptibility to protease 401, and thermal **stability** did not differ appreciably between wild type and mutant enzymes. In the overall transamination between aspartate and 2-oxoglutarate, V17A represented a typical Km mutant while V17I retained the substrate binding affinity fairly well. In contrast, replacement of Phe18 by Ala resulted in a large decrease in both catalytic rate and binding affinity for substrates. F18W, F18Y, and F18H showed a moderate decrease in kcat and a considerable increase in Km values. Single-turnover reactions with four individual substrates yielded analogous results to those obtained for the overall reaction and, in addn., revealed that k/Kd values of mutants F18A and F18H were over 10 times lower for C5 substrates (glutamate and 2-oxoglutarate) than those for C4 substrates (aspartate and oxalacetate). All mutant enzymes showed variously increased Kd values for substrate analogs such as 2-methylaspartate, succinate, and glutarate. 1H NMR observations of F18H, in which His18 served as a built-in probe, were in accord with the behavior that would be expected from the conformational transition. The authors conclude that, although Val17 and Phe18 may not be essential for catalysis, the presence of a bulky residue of appropriate size at each position is crit. for productive binding of substrate.
AN 121:173879 CA
TI Functional role of the amino-terminal mobile segment in catalysis by porcine cytosolic aspartate aminotransferase. Critical importance of Val17 and Phe18 for productive binding of substrates
AU Nishimura, Kosuke; Higaki, Tsuyoshi; Okamura, Hitoshi; Tanase, Sumio
CS Sch. Med., Kumamoto Univ., Kumamoto, 860, Japan
SO J. Biol. Chem. (1994), 269(40), 24712-18
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L6 ANSWER 4 OF 4 MEDLINE
AB The functional roles of Val37 and Gly38 in porcine cytosolic aspartate

aminotransferase have been studied in the site-directed mutants V37A, G38A, and G38S where the size and hydrophobic character of these residues has been altered. Previous x-ray studies have shown that Val37 and Gly38, which are part of a flexible loop, interact directly with bound substrate.

From x-ray and solution experiments we find that the V37A, G38A, and G38S mutations do not cause significant perturbations to the unliganded enzyme.

Replacing Val37 with a less bulky alanine residue does not affect the maximal catalytic rate (kcat), but it does increase significantly the Michaelis constants for substrates in the overall transamination reaction between aspartate and 2-oxoglutarate. On the other hand, replacing Gly38 with alanine or serine results in striking decreases in kcat to 5 and 0.6%, respectively, of the value observed for the wild-type enzyme, as well as in considerable increases in Km values. Consequently, the catalytic competence, kcat/Km, decreases by 3 orders of magnitude for G38A

and by 4 orders of magnitude for G38S. Single turnover reactions of G38A and G38S with four individual substrates (aspartate, glutamate, oxalacetate, and 2-oxoglutarate) are characterized by kinetic parameters that are largely consistent with those of the overall reaction. In addition, the mutations at position 38 impair more seriously the catalytic

competence of the enzyme toward C5-substrates than toward C4-substrates. We conclude that Gly38 is probably required for proper function of the enzyme because it permits a high level of flexibility for the 36-39 peptide, which in turn allows the essential substrate-induced movement of the small domain.

AN 94043333 MEDLINE
DN 94043333 PubMed ID: 8227035
TI Functional roles of **valine** 37 and glycine 38 in the mobile loop of porcine cytosolic **aspartate aminotransferase**.
AU Pan Q W; Tanase S; Fukumoto Y; Nagashima F; Rhee S; Rogers P H; Arnone A; Morino Y
CS Department of Biochemistry, Kumamoto University School of Medicine, Japan.
NC GM-40852 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Nov 25) 268 (33) 24758-65.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199312
ED Entered STN: 19940117
Last Updated on STN: 19980206
Entered Medline: 19931220

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